

Antibacterial Effect of *Acronychia Pedunculata* Fresh Extract against *Staphylococcus Aureus*: A Study in Vitro

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ABSTRACT

With reference to the text series of *Talpatepiliyam*, it is mentioned that leaves and stem bark of *Ankenda* (*Acronychia pedunculata*) are used externally in all types of *oduvana* and *gadu*. *Staphylococcus aureus* is the common cause of skin infections such as abscesses and wounds. This study was aimed to investigate whether there is antibacterial activity of fresh extracts of stem bark, leaves and combined sample (stem bark and leaves) of *Acronychia pedunculata* grown in Sri Lanka against *S. aureus* (ATCC25923) and Minimum Inhibitory Concentration (MIC) was studied. Kirby Baurer Well diffusion method of Antibacterial Susceptibility Testing was used to determine antibacterial activity of each fresh extract. Amoxicillin (10mg/ml) and distilled water were used as positive and negative control respectively. Zone diameters were interpreted and data were statistically analyzed. The results showed that there was antibacterial effect of each sample. The highest zone diameter was observed for the combined sample (inhibition zone: 11.67mm). This could be due to the synergetic activity of each compounds from each plant material used. Fresh extracts of stem bark showed the second most zone diameter (11mm) while fresh extracts of leaves the least (9mm). As fresh extracts of combined sample showed the highest activity, dilution series for MIC were prepared from the combined sample. The MIC of combined sample for the growth of *S. aureus* was 10^{-2} mg/ml. This study could be further investigated for more gram positive and negative bacterial and fungal species.

KEYWORDS: *Acronychia pedunculata* fresh extract, *Staphylococcus aureus*, Minimum Inhibitory Concentration

1. INTRODUCTION

Background of the Research

According to World Health organization, medicinal plants would be the best source to obtain a variety of drugs (WHO, 2016). For thousands of years, natural products have been used in traditional medicine all over the world to treat various diseases and disorders. Medicinal plants generally contain various compounds which may be a potential natural antimicrobial combination and which will serve as an alternative, effective and safe antimicrobial treatment (Monecke et al, 2011). The past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Gremaet et al, 2015). The present day antibiotics have their own drawbacks and are also losing their efficiency to resist against pathogens. Consequently, the research areas have shifted their interest in obtaining newer compounds from plants which are safer and more efficient against the ever growing resistant pathogens.

With reference to the text series of *Talpatepiliyam*, it is mentioned that leaves and stem bark of *Ankenda* are used in all types of *oduvana* and *gadu*¹ (TP, 1994). In Sri Lankan Ayurveda Pharmacopoeia, it is stated that *Acronychia pedunculata* "*Ankenda*" having *amla*, *kashaya*, *tikta rasa*,

¹ TP – *Talpatepiliyam*

ruksha and *ushnaguna*, *amla* or *kativipaka*, *ushnavirya*, action of *vatakaphahara*, externally leaves and stem bark are *shothahara* and *vimlapana*, internally *vatanulomana*, *antarvidradhihara* and *raktashamaka*, used in formulations of *nilyaditaila*, *ankendataila*, *rata taila*² (AP, 1979). In traditional and folk medicine of Sri Lanka, the bark is used externally on swelling, fractures, intestinal infections, sores and tonic for scabies and ulcers. The bark has also been used internally as a purgative (Jayasinghe et al, 2006).

Bacteria causing cutaneous abscesses are typically indigenous to the skin of the involved area. Although *Staphylococcus aureus* is not always pathogenic, it is the common cause of skin infections such as abscesses and wounds (Cooper et al, 2004).

Previous researches have revealed that boiled aqueous extracts of dried *Acronychia pedunculata* possess moderate antibacterial effect against *Staphylococcus aureus* and no any antibacterial effect against *Escherichia coli* at any concentration (Ranaweera et al., 2016). Methanol, chloroform, petroleum ether, ethyl acetate extracts of *Acronychia pedunculata* were tested against *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* (Kanerva et al, 2011).

² AP - Ayurveda Pharmacopoeia

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Hence, based on the available data regarding the usage of medicinal values of this species, the present study is aimed at investigating the antibacterial effect of fresh extracts of stem bark and leaves of *Acronychia pedunculata* against laboratory specimens of *Staphylococcus aureus* as this is mainly used externally in Ayurvedic and traditional system of medicine.

Aim and Objectives of the Research:

General Objectives

To Evaluate the antibacterial effect of fresh extracts of stem bark, leaves and stem bark and leaves of *Acronychia pedunculata* against laboratory specimens of *Staphylococcus aureus*.

Specific Objectives

1. To calculate the mean inhibitory zone diameter of fresh extracts of stem bark, and leaves of *Acronychia pedunculata* against the laboratory specimens of *Staphylococcus aureus*.
2. To evaluate the Minimum Inhibitory Concentration of fresh extracts of stem bark and leaves of *Acronychia pedunculata* against the laboratory specimens of *Staphylococcus aureus*.

2. METHODOLOGY

2.1. Collection and Authentication of the plant

Acronychia pedunculata plants with stem bark and leaves were collected from the garden of Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya.

They were selected according to *sharangadharasamhitā*.

The botanical identification was authenticated through macroscopic appearance according to Ayurveda Pharmacopoeia and specimen collection of plant museum of Department of *Dravyaguna Vignana*, Gampaha Wickramarachchi Ayurveda Institute (GWAI), University of Kelaniya, Yakkala, Sri Lanka.

2.2. Preparation of fresh extract

Raw Leaves and Stem bark of *Acronychia pedunculata* were cleaned and washed well with distilled water. Fresh extract was obtained by using the water content possessed by plant itself without adding extra water.

Sample A-

60g of stem bark of *Acronychia pedunculata* were measured, cleaned well and crushed. It was squeezed and filtered with a clean cloth without leaving particles.

Sample B-

60g of leaves of *Acronychia pedunculata* were measured, cleaned well and crushed using. It was squeezed and filtered with a clean cloth without leaving particles.

Sample C -

60g of stem bark and 60g of leaves of *Acronychia pedunculata* were measured, cleaned well and crushed. It was squeezed and filtered with a clean cloth without leaving particles.

Prepared specimens were used for the antibacterial assay immediately without leaving for contaminations.

2.3. Anti-bacterial assay

Antibacterial activity of fresh extracts were evaluated using the agar well diffusion method as described by Clinical and Laboratory Institute³ (NCCLS, 2003). Distilled water was

³ NCCLS - National Committee for Clinical Laboratory Standards.

used as the negative control and Amoxicillin (10mg/ml) as the positive control.

The growth medium used for this experiment was Muller Hinton Agar and the antibacterial activities were determined against *Staphylococcus aureus* (ATCC 25923). Wells were prepared by 6mm sterile cork borer. 50µl of prepared fresh extracts, 50µl of prepared amoxicillin as the positive control and 50µl of distilled water as the negative control were introduced into the wells.

Incubation temperature was 37°C and incubation period was 24 hours. At the end of the incubation period the diameter of the inhibition zone around each well was measured (in mm) using a Vernier caliper. All experiments were conducted in triplicate.

2.4. Evaluation of Minimum Inhibitory Concentration (MIC)

The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism is known as the MIC. The fresh extract of the combined sample that demonstrated effective antibacterial activities against *S. aureus* were subjected to the determination of MIC using Dilution method as described by National Committee for Clinical Laboratory Standards (NCCLS, 2003).

3. RESULTS AND DATA ANALYSIS

3.1. Results of Antibacterial Susceptibility Test

Table 1: Results of Antibacterial Susceptibility Test

Sample	Zone diameters (mm)				
	A	B	C	Positive Controller	Negative Controller
1	12	9	11	40	0
2	10	8	12	41	0
3	11	10	12	40	0

A. Fresh extract of stem bark

B. Fresh extract of leaves

C. Fresh extract of leaves and stem bark

Positive Controller- Amoxicillin

Negative Controller- Distilled water

3.2. Descriptive Statistics of tested samples for Antibacterial Susceptibility Test.

Table 2: Descriptive Statistics of tested samples for Antibacterial Susceptibility Test

Sample	N	Minimum	Maximum	Mean
Fresh extract of stem bark	3	10	12	11.00
Fresh extract of leaves	3	8	10	9.00
Fresh extract of stem bark and leaves	3	11	12	11.67
Amoxicillin	3	40	41	40.33

3.3. Results of Minimum Inhibitory Concentration of combined sample

Table 3: Results of Minimum Inhibitory Concentration.

Sample	Zone diameters (mm)					Positive Controller	Negative Controller
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³			
1	12	9	7	0		41	0
2	11	8	7	0		41	0
3	11	7	6	0		40	0

10^0 - 10^0 concentration of fresh extract of leaves and stem bark (combined sample)

10^{-1} - 10^{-1} concentration of fresh extract of leaves and stem bark

10^{-2} - 10^{-2} concentration of fresh extract of leaves and stem bark

10^{-3} - 10^{-3} concentration of fresh extract of leaves and stem bark

Positive Controller- Amoxicillin

Negative Controller- Distilled water

So according to the results,

The lowest concentration of fresh extract of stem bark and leaves that will inhibit the growth of *Staphylococcus aureus*; Minimum Inhibitory Concentration is 10^{-2} mg/ml.

4. DISCUSSION

4.1. DISCUSSION

All fresh extracts of *Acronychia pedunculata* showed antibacterial activity against gram positive *Staphylococcus aureus* (ATCC 25923) strains. Fresh extracts of stem bark and leaves showed the highest antibacterial activity (inhibition zone diameter; 11.67mm) while Fresh extracts of stem bark showed the second most (11mm) and Fresh extracts of leaves the least (9mm). The positive controller was Amoxicillin and the negative controller used was distilled water. Amoxicillin showed a diameter of 40.33mm and distilled water showed no zone diameter. The minimum concentration of which the fresh extracts of combined sample inhibit the growth of *S.aureus* is 10^{-2} mg/ml. Previous researches have conducted to investigate boiled water extracts of *Acronychiapedunculata* against *staphylococcus aureus* using Disk diffusion antibacterial susceptibility testing method. The highest activity observed for the combined boiled sample which had all ingredients (roots, stem barks, leaves, flowers and seeds) in the same boiled aqueous solution and the antibacterial activity was observed in a concentration dependent manner. Lowest activity was recorded for seeds while root and stem bark boiled aqueous solution showed second most and third most antibacterial activity against *S.aureus* (ATCC 25923) on a concentration dependent manner (Ranaweera et al., 2016).

Antibacterial potential in vitro of hexane, chloroform and methanol extracts made from leaves, stem bark, flowers, seeds or roots of Sri Lankan grown *Acronychia pedunculata* plant against *Staphylococcus aureus* (ATCC25923) using agar disc diffusion bioassay technique. All the three flower extracts exerted marked antibacterial activity against *S. aureus*. The overall order of potency against *S. aureus* was chloroform flowers> chloroform seeds > hexane leaves > chloroform leaves> methanol flowers>hexane flowers> methanol seeds (Ranaweera et al., 2016).

In this study, we investigated *in vitro* antibacterial activity of the fresh extract with the water content in the plant itself, without adding extra amount of water. The effect is depending on the plant part and the type of the solvent used in the extraction process. Here the hydrophilic phytoconstituents of *Ankenda* were evaluated for the antibacterial activity against *Staphylococcus aureus*.

4.2. CONCLUSION

The results of this study showed that fresh extracts of *Acronychia pedunculata* found in Sri Lanka possess

antibacterial properties against *S.aureus* (ATCC 25923) strain. At the same time this study rationalizes the use of this plant in traditional and folk medicine treatments in Sri Lanka.

This could be further investigated for more gram positive and gram negative bacterial and fungal species. It is worth to investigate each extract individually using various chromatographic techniques and identify active compounds separately. This could be developed as a clinical research for the patients with chronic wounds as it is mentioned that fresh extracts of leaves and stem bark of *Acronychia pedunculata* was used for *oduvana* and *gadu* in the text *Talpatepiliyam*.

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Figure 1: Zone diameters of sample 1- ABST



Figure 2: Zone diameters of sample 2- ABST



Figure 3: Zone diameters of sample 3 - ABST

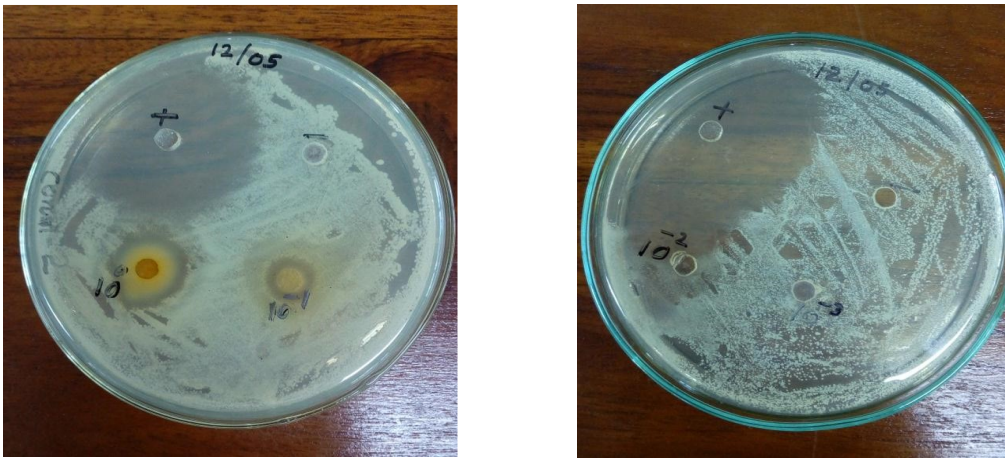


Figure 4: Zone diameters of sample 1- MIC

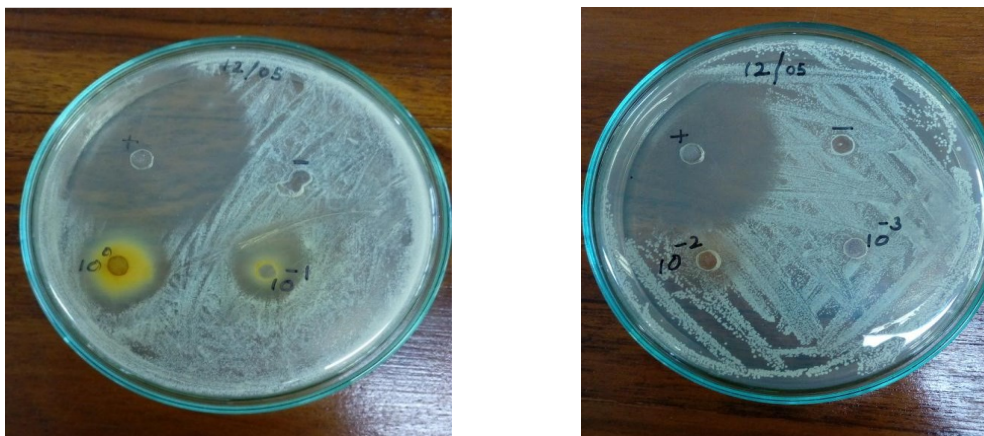


Figure 5: Zone diameters of sample 2 - MIC

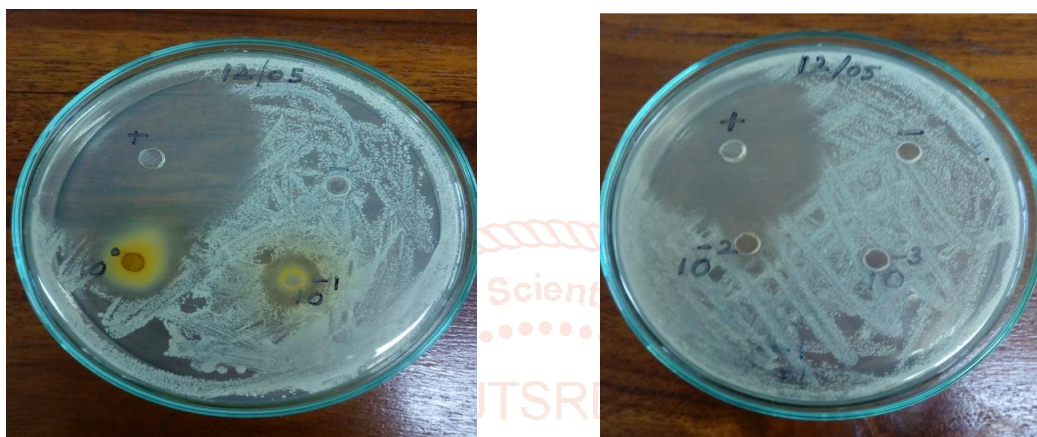


Figure 6: Zone diameters of sample 3 - MIC